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(57) Abstract

The present invention relates to new pharmaceutical compositions comprising a spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active substances, the process for their production, and the use of same in surgery and in particular in microsurgery.

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Pharmaceutical compositions comprising a spongy material consisting of ester derivatives of hyaluronic acid combined with other pharmacologically active substances

Field of the invention

The present invention relates to new pharmaceutical compositions comprising a spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active substances, the process for their production, and the use of same in surgery and in particular in of microsurgery.

Description

As known, hyaluronic acid plays a major role in tissual repair processes, especially in the early granulation tissue formation phases, by serving several functions: it stabilizes the coagulum matrix and controls the degradation of same, helps response of inflammatory cells, e.g. polymorphonucleates and monocytes, of mesenchymal cells, e.g. fibroblasts and endothelial cells, and orients the successive migration of epithelial cells.

As known, the administration of hyaluronic acid solutions speeds up the recovery of patients suffering from decubitus ulcers, wounds and burns.

The role of hyaluronic acid (HA) during the various tissual repair process phases was illustrated through a theoretical model by Weigel P.H. et al., "A model for the role of hyaluronic acid and fibrin in

the early events during the inflammatory response and wound healing", J. Theor. Biol., 119, 219, 1986.

The main problem still demanding solution is that repeated HA administrations are required, whatever the vehicle used, HA being very rapidly eliminated from the lesion site.

Should HA solutions be directly applied, no drug release control would be possible. This would cause short times of drug retention by the lesion and, consequently, repeated administrations resulting in the treated area moistening and maceration, would be required.

Furthermore, should non-perfectly biocompatible inert matrices be used, local phlogistic reactions and cicatrix adhesions would develop.

It has now been found that the new pharmaceutical compositions forming the object of the present invention - compared with the compositions already known - represent a significant technological improvement in that they do not raise the same problems and give better results.

The compositions of this invention are made of a spongy material consisting of total or partial ester derivatives of hyaluronic acid, wherein a solution containing a compatible active ingredient is absorbed and later co-lyophilized.

Said new compositions acquire greater flexibility and softness by addition of glycerin or appropriate plasticizers.

In another embodiment of the present invention, a pierced biocompatible membrane capable of favouring cell growth adheres to

one or both sides of the colyophilized pharmaceutical composition.

Other objects of the present invention are a process for the production of said compositions and the use of same in surgical and in particular microsurgical practice.

The claimed compositions represent a great technological progress, being capable of acting as a mechanical guide for re-epithelization thanks to the chemo-physical characteristics of the spongy material and to the presence of active ingredients absorbed therein and, at the same time, of providing a controlled drug release at the site of treatment. Consequently, high local drug concentrations and slow release of same are guaranteed.

Due to the presence of hyaluronic acid in the absorbed and colyophilized solution, the new compositions combine, in one product, the capability of HA to induce a rapid and complete tissual repair process and the characteristics of applicability, elasticity, and tolerability of hyaluronic acid ester derivatives, which are excellent mechanical guides for the tissual repair process.

Furthermore, the biocompatibility of the spongy material and the pharmacological activity of the hyaluronic acid absorbed therein suggest that the new compositions are an ideal biomaterial for use in various surgical fields, such as for example otologic and otoneurologic microsurgery, functional, post-traumatic and endoscopic rhinosinusal microsurgery, plastic and reconstructive surgery, and any other surgical practice envisaging the use of non-

reabsorbable materials, such as controlled release systems of pharmacologically active substances.

The new compositions allow maintaining high local concentrations of the active ingredient, e.g. hyaluronic acid, at the site of treatment and offer the great advantage of a single administration, which results in a reduction in the number of physicians' interventions, dispensary controls, and hospitalizations.

The new compositions have a constitution guaranteeing a solid matrix of optimal elastic and biocompatible properties, capable therefore, of acting as a mechanical guide for tissual repair processes in general and for the tympanic membrane repair process in particular.

Addition of glycerin or other appropriate plasticizers to the claimed compositions gives a flexible and soft spongy material, which offers two further advantages:

- ease of handling and application to the site of treatment, the material softness making the application less painful;
- highly increased hydration, the spongy material absorbing in water about 10 times its original weight in 3 to 4 seconds.

In another embodiment of the present invention, a pierced biocompatible membrane capable of favouring cell growth on its surface, e.g. fibroblasts and keratinocytes, is applied to the surface of the pharmaceutical compositions to be placed in contact with the lesion.

The pharmaceutical compositions of this invention are made of a

spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, in particular HA ethyl ester (HYAFF-7) and HA benzyl ester (HYAFF-11), which is caused to absorb a solution containing hyaluronic acid or another pharmacologically active ingredient (e.g. growth factors, fungicides, antibiotics, bacteria-fighting compounds, steroid and non-steroid anti-inflammatory agents, etc.) and in particular pharmacologically active hyaluronic acid derivatives as are illustrated in European patent applications EPA 0216453 and EPA 0197718 filed in the name of Fidia S.p.A., which are then subjected to lyophilization.

With a view to obtaining an end product of improved elasticity and softness, glycerin or an appropriate plasticizer may be added before final lyophilization.

The characteristics of the end product may vary depending on the HA ester derivatives solution used to produce the spongy material and on the absorbed solutions.

Said characteristics are summarized in Table 1.

TABLE 1

Description	Unit of measurement	Lower limit	Upper limit
Aspect: Odourless white sponge			
Dry weight	mg/cm^3	30	200
Water absorption	% (w/w)	500	1500
IR identification	-	positive	positive
Esterification	%	50	102
HA content	% (w/w)	3	50
LAL test	UE/mg	-	0.2
Glycerin (optional)	% (w/w)	5	30

In another embodiment of this invention, the pierced membranes applied to one surface of the spongy material are biocompatible and made of materials of natural, synthetic or semisynthetic origin, preferably of HA benzyl ester, and favour the growth on their surface of cells, such as for example fibroblasts and keratinocytes.

In particular, the membranes that may be used are 10 to 500 μ thick and pierced with a regular series of holes of a definite and constant size between 10 and 1000 μ, separated from each other by a constant distance of between 50 and 1000 μ, as are illustrated in European patent application EPA 91108654.4 filed on 28th May, 1991, in the name of Fidia S.p.A.

With a pierced membrane applied to the surface of the spongy material, the new compositions combine their aforementioned

advantages with the specific action of pierced membranes, i.e. they also favour re-epithelization.

The products of this invention are obtained on the basis of the following process.

1) Solubilization

The starting material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, is completely solubilized in an appropriate solvent to a concentration of 20 to 50 mg/ml, preferably 35 mg/ml. The solution obtained is filtered through a filter with 40 µm pores.

2) Coagulation

The resulting solution is poured into appropriate containers, later placed in a chamber with relative humidity of 60 to 100%, preferably 85%, until evident coagulation of the material.

3) Washing

The solid panels obtained are cut into lumps of appropriate size, which are placed in a NaCl solution at a concentration of 80 to 120 g/l, preferably 100 g/l. Said solution is periodically renewed.

4) Lyophilization

Lyophilization is carried out as follows:

4.1) Lumps are placed on freeze-dryer plates.

4.2) Starting from room temperature, plates temperature lowering is set to -45°C. The temperature lowering rate is the maximum admitted by the system.

4.3) Plates are cooled to the freezing temperature and maintained at

said temperature for a period of 3 hrs so the lumps can be cooled to said temperature.

4.4) In-chamber pressure is set to 3×10^{-1} to 2×10^{-1} bar and heating is started. The heating temperature is -12°C; said temperature has to be reached gradually over a period of 4 hrs and maintained at said value for 35 to 55 hrs, preferably 48 hrs, i.e. the time required for complete sublimation.

4.5) Temperature rise is then set to 20°C, which temperature is reached over a period of 12 hrs and maintained at said value for 3 hrs.

5) Washing

The resulting panels are placed in a demineralized and apyrogenous water bath and washed for at least 16 hrs; during said step, baths are periodically renewed every 2 or 4 hrs.

6) Imbibition with active ingredient solution

Lumps are imbibed with the solution containing drug at a concentration of from 0.1% to the limit of solubility of the solute. Wishing to obtain soft and flexible sponges, glycerin or an appropriate plasticizer is added to the solution in an amount of 5 to 30% by wt., preferably 20%.

7) Drying by lyophilization

An additional lyophilization cycle as described under 4) is carried out.

The products obtained may be sterilized by gamma-rays or equivalent systems.

The following examples illustrate the process for the preparation of the products of this invention. These examples are illustrative only; in no event are they to be regarded as limiting the scope of the invention.

Example 1

Method for the preparation of HYAFF-7 ovoid spongy tampons having diameters of 15 mm x 10 mm and thickness of 4 mm, each containing 10 mg hyaluronic acid

40 g of HYAFF-7 were solubilized in DMSO (1142 ml) in a reactor equipped with agitator, thermostabilized at 25°C.

Once the product solubilization was completed, i.e. after 8 hrs approx., the solution was filtered through a membrane with pores of 40 µm. The solution was poured onto a 30 x 45 cm stainless steel tray.

The tray was placed in a chamber under 25°C temperature control, saturated with steam acting as coagulating solvent. Coagulation lasted 60 hrs approx.

A gelatinous HYAFF-7 cake was obtained. For ease of handling, it was cut into 100 x 150 mm lumps, which were placed in a saline solution (2000 ml) at a concentration of 100 g/l of NaCl for a period of 3 days.

The saline solution baths were renewed every 4 hrs.

Lumps were placed on the plates of a pre-set freeze-dryer to be subjected to a lyophilization cycle.

Lyophilization was carried out as follows:

- starting from room temperature, plates temperature lowering was set to -45°C at the maximum lowering rate admitted by the system;
- plates were cooled to the freezing temperature and maintained at said temperature for 3 hrs so the lumps could be cooled to said temperature;
- in-chamber pressure was set to 3×10^{-1} to 2×10^{-1} bar and heating was started. The heating temperature was -12°C; said temperature had to be reached gradually over a period of 4 hrs and maintained at said value for 48 hrs approx. until sublimation completion;
- temperature rise was then set to 20°C, which temperature was reached over a period of 12 hrs and maintained at said value for 3 hrs.

The spongy product thus obtained was washed 6 times with distilled apyrogenous water (1000 ml) for NaCl elimination. Each washing lasted 4 hrs approx.

Lumps having diameters of 15 cm x 10 cm and thickness of 5 mm were hollow punched to obtain approx. 300 oval tampons with diameters of 15 mm by 10 mm.

A hyaluronic acid solution (150 ml) at a concentration of 24 mg/ml was prepared in an appropriate reactor.

Each tampon was wrung out to remove most wash water. Then 412 µl of the previously prepared solution, corresponding to 10 mg of hyaluronic acid, were distributed on one tampon side by a dosing system.

The time taken for the solution complete absorption and spreading inside the spongy structure was 30 min.

The soaked tampons were further lyophilized as per the parameters of the previous cycle until obtaining the end product.

Example 2

Method for the preparation of HYAFF-7 ovoid spongy tampons having diameters of 15 mm x 10 mm and thickness of 4 mm, each containing 10 mg of hyaluronic acid, whereto an adhesive HYAFF-11 film pierced with constantly spaced (80 µm) holes of 40 µm size is applied

Some tampons prepared as per Example 1 were made to adhere to a film pierced with constantly spaced (80 µm) holes of 40 µm size according to the following procedure.

Pierced film sheets (120 x 120 mm) were cut into pieces of 20 x 25 mm size. Meanwhile, a solution of HYAFF-7 in hexafluoro isopropanol (HFIP) at a concentration of 20 mg/ml was prepared in an appropriate reactor. Once the solubilization was completed, the solution was filtered through a membrane with pores of 40 µm.

Five 15 µl drops of a HYAFF-7 solution in HFIP were distributed on one tampon side by a suitable dosing system as follows: 4 drops at the end points and 1 drop at the central point.

The tampon side where the five drops were distributed was caused to adhere to the centre of the pierced film by applying a slight pressure.

Fifteen minutes later, i.e. the time required for the low-boiling solvent to evaporate, a perfect adhesion between film and tampon was

obtained.

Once cohesion was completed, tampons were allowed to stand in an oven at a temperature of 30°C and at a pressure of 1×10^{-2} mbar for a period of 24 hrs.

Example 3

Method for the preparation of HYAFF-7 spongy tampons, flexible and dry-mouldable in ovoid form, having diameters of 15 mm x 10 mm and thickness of 4 mm, each containing 10 mg of hyaluronic acid

No. 6 lumps of spongy material having dimensions of 150 mm x 100 mm and 5 mm thickness were prepared as per Example 1 until the stage of material washing with NaCl, after the first lyophilization cycle.

1000 ml of glycerin in distilled and apyrogenous water at a concentration of 8% were prepared separately.

Once the washings were completed, the 6 lumps were wrung out by a mechanical system to remove most of the absorbed water and placed in the glycerin solution previously prepared. Spongy lumps were allowed to stand in said solution for approx. 60 min.

The process proceeds as per Example 1.

A glycerin content of 20% was detected by chemical analysis.

Example 4

Preparation of a spongy material consisting of 60% HYAFF-7 and 40% HYAFF-11, containing 10 mg hyaluronic acid

A solution of HYAFF-7 (24 g) and HYAFF-11 (16 g) in DMSO (1142 ml) was obtained by mixing in a reactor equipped with a vacuum/pressure system and agitator, and thermostabilized at 25°C.

Once solubilization was completed, the solution was filtered through a membrane with pores of 40 µm.

The process proceeds as per Example 1.

Some in vivo tests were carried out with a view to proving the efficacy of the compositions of the invention.

The results of a test made to evaluate the efficacy of the new compositions in favouring the tympanic membrane repair process in the rat are reported below.

TEST 1

With a view of evaluating the efficacy of the new compositions in favouring the tympanic membrane repair process in the rat and the time of repair, a test was conducted using the diabetic rat as an experimental model.

Eight mature rats (T, D, C, TD, TC, TDC, B, GAD) aged 8 months and weighing 250-350 g, with six-months' diabetes induced by treatment with streptozotocine (STZ, 60 mg/kg i.p.) were subjected to bilateral tympanic membrane perforation.

The upper-posterior quadrant of the tympanic membrane (TM) of the left ear was bilaterally perforated by means of a lanceolate bistouri with the aid of an operating microscope.

The TM was dressed with a tampon obtained as per Example 1 and soaked with one drop of physiological saline solution. The tampon was fixed therein by a cross stitch sewn on the external acoustic meatus.

The TM of the right ear was not treated and was used as a control. A

cross stitch was sewn also on the external acoustic meatus of the right ear.

Tampons were left in situ for a period of 6 days; during said period two external observations were conducted to make sure that dressings and stitches were regularly in place. All dressings were removed on the 6th day.

TM controls with a microscope were made on the 6th, 8th, 10th, 12th, and 15th day.

Complete repair of the left TM was observed on the 6th day in rats D, TC and B; on the 8th day in two further rats, i.e. C and GAD; on the 10th day in the three remaining rats, i.e. T, TD and TDC. Always on the 10th day, complete repair of the right ear TM was observed in rats D and C; on the 12th day in rats TC and B; on the 15th day in the remaining four rats T, TD, TDC, and GAD.

The results obtained are recapitulated in Table 2.

TABLE 2

rat	T		D		C		TD		TC		TDC		B		GAD	
ear	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
day																
6th			*						*				*			
8th					*										*	
10th	*			X		X	*				*					
12th										X				X		
15th		X						X				X				X

* = complete repair of the left tympanic membrane (TM)

X = complete repair of the right tympanic membrane (TM)

Briefly, the control made on the 10th day showed that all TM's treated with the new compositions were repaired , while only two untreated TM's showed the same result. Furthermore, on the last observation through an operating microscope, the TM's repaired with the new compositions showed improved characteristics of gloss and transparency, no tympanic retraction, dyschromia, and dysmorphism.

To conclude, the new compositions proved to be effective in favouring an improved TM repair in much shorter times than required by spontaneous repair.

The animal model selected for the experiment, i.e. the rat aged 8 months and with 6 months' diabetes, implied the hardest experimental conditions: as known, in fact, said animals exhibit noticeably slowed down tissual repair processes as a consequence of the induced dysmetabolic pathology. Said hard experimental conditions were even more evident by the long diabetic condition (6 months).

Therefore, the results obtained provide evidence that the new compositions are highly effective in inducing a complete and very rapid tissual repair, even by a single administration and with a few days' contact with the damaged TM. The experimental results obtained by us suggest that the new compositions can be profitably used in surgery and, in particular, microsurgery as well as in the treatment of tympanic membrane perforations.

Furthermore, the biocompatibility characteristics of the spongy material and the pharmacological activity of the hyaluronic acid absorbed therein make the new compositions an ideal biomaterial for use in various surgical fields, such as for example otologic and otoneurologic microsurgery, functional, post-traumatic and endoscopic rhinosinusal microsurgery, plastic and reconstructive surgery, and any other surgical practice envisaging the use of non-reabsorbable materials, such as controlled release systems of pharmacologically active substances suitable for favouring the tissual repair process.

Furthermore, since the spongy material can absorb solutions containing pharmacologically active ingredients, either singly or as a mixture with HA or in the form of HA salts or esters, such as e.g. antibiotics, fungicides, bacteria-fighting compounds, growth factors, corticosteroids, non-steroid anti-inflammatory agents, as are e.g. illustrated in European patent applications EPA 0216453 and EPA 0197718 in thename of Fidia S.p.A., it is possible to obtain a wide range of highly interesting products to be used in external dressings, endocavitary and post-operative dressings.

Some examples of the possible applications of the compositions of the invention are conveyed hereinbelow by way of indication, not of limitation.

- A product capable of releasing HA and an antibiotic at the site of treatment can be used, e.g., for dressing infected wounds, cutaneous ulcers and surgical wounds and for treating external

otitides, bacterial vaginites, etc.

- A combined release of HA and a fungicide is greatly advantageous in the treatment of skin mycoses in general and of external acoustic duct mycoses in particular, an adequate ad hoc local treatment being possible.

- A combined release of HA and a corticosteroid is greatly advantagesous in the treatment of eczematous dermatitises and of all dermatologic pathologies favourably affected by local treatment with corticosteroids. A particular application concerns the eczematous dermatitises of the external acoustic duct.

- A combined release of HA and growth factors finds application in plastic and reconstructive surgical practices, whenever cellular growth and superficial and deep tissues reconstruction are to be favoured and enhanced.

CLAIMS

1. Pharmaceutical compositions comprising a spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly of as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active ingredients.

2. The pharmaceutical compositions according to claim 1, wherein said hyaluronic acid esters are ethyl ester or benzyl ester.

3. The pharmaceutical compositions according to claim 1, wherein said solution contains hyaluronic acid or one of its salts or derivatives and/or other pharmacologically active ingredients either singly or as a mixture therof.

4. The pharmaceutical compositions according to claim 1, wherein said active ingredients of said solution exert an antibiotic, antimycotic, antibacterial, anti-inflammatory action and/or enhance cellular growth and tissual repair or reconstruction.

5. The pharmaceutical compositions according to claim 1, wherein glycerin is present.

6. The pharmaceutical compositions according to claim 1, wherein a biocompatible pierced membrane of natural, synthetic or semisynthetic origin favouring cells growth on its surface is applied to the spongy material surface or surfaces to be placed in contact with the lesion.

7. The pharmaceutical compositions according to claim 6, wherein said biocompatable membrane is from 10 to 500 μ thick and is pierced with a regular series of holes of a definite and

constant size of between 10 and 1000 μ, separated from each other by a constant distance of between 50 and 1000 μ.

8. The pharmaceutical compositions according to claims 6 and 7, wherein said biocompatible pierced membrane consists of hyaluronic acid benzyl ester.

9. Process for the preparation of new pharmaceutical compositions comprising a spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active ingredients, via the following steps:

1) Solubilization

The starting material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, is completely solubilized in an appropriate solvent to a concentration of 20 to 50 mg/ml, preferably 35 mg/ml. The solution obtained is filtered through a filter with 40 μm pores.

2) Coagulation

The resulting solution is poured into appropriate containers, later placed in a chamber with relative humidity of 60 to 100%, preferably 85%, until evident coagulation of the material.

3) Washing

The solid panels obtained are cut into lumps of appropriate size, which are placed in a NaCl solution at a concentration of 80 to 120 g/l, preferably 100 g/l. Said solution is periodically renewed.

4) Lyophilization

Lyophilization is carried out as follows:

4.1) Lumps are placed on the freeze-dryer plates.

4.2) Starting from room temperature, plates temperature lowering is set to -45°C. The temperature lowering rate is the maximum admitted by the system.

4.3) Plates are cooled to the freezing temperature and maintained at said temperature for a period of 3 hrs so the lumps can be cooled to said temperature.

4.4) In-chamber pressure is set at 3×10^{-1} to 2×10^{-1} bar and heating is started. The heating temperature is -12°C; said temperature is to be reached gradually over a period of 4 hrs and maintained at said value for 35 to 55 hrs, preferably 48 hrs, i.e. the time required for complete sublimation.

4.5) Temperature rise is set to 20°C, which temperature is reached over a period of 12 hrs and maintained at said value for 3 hrs.

5) Washing

The resulting panels are placed in a demineralized and apyrogenous water bath and washed for at least 16 hrs; during said step, baths are periodically renewed every 2 or 4 hrs.

6) Imbibition with active ingredient solution

Panels are imbibed with the solution containing drug at a concentration of from 0.1% to the solubility limit of the solute.

Wishing to obtain soft and flexible sponges, glycerin or an appropriate plasticizer is added to the solution in an amount of 5 to 30% by wt., preferably 20%.

7) Drying by lyophilization

A second lyophilization cycle as per point 4 is carried out.

10. Use in medical practice of pharmaceutical compositions comprising a spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly of as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active ingredients.

11. The use according to claim 10, in surgery and/or microsurgery.

12. The use according to claim 10, wherein surgery is plastic or reconstructive surgery.

13. The use according to claim 10, wherein microsurgery is otologic or otoneurologic microsurgery, in particular for the treatment of tympanic membrane lesions, and functional, post-traumatic and endoscopic rhinosunusal microsurgery.

14. The use according to claim 10 in external dressings, endocavitary and post-operative dressings.

15. Use in medical practice of pharmaceutical compositions comprising spongy material consisting of total or partial ester derivatives of hyaluronic acid, either singly or as a mixture thereof, co-lyophilized with a solution containing other pharmacologically active ingredients, combined with a biocompatible pierced membrane of natural, synthetic or semisynthetic origin favouring cells growth on its surface, which is applied to the spongy material surface and surfaces to be placed in contact with the lesion.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 94/00294

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61L27/00 A61L15/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61L C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP,A,0 526 865 (FIDIA S.P.A.) 10 February 1993 see examples	1-4
Y	EP,A,0 462 426 (FIDIA S.P.A.) 27 December 1991 cited in the application see page 3, line 31 - line 41; examples	1-15
Y	EP,A,0 216 453 (FIDIA S.P.A.) 1 April 1987 cited in the application see claims	1-15
P,Y	WO,A,94 01468 (M.U.R.S.T.) 20 January 1994 see the whole document	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 94/00294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 197 718 (FIDIA S.P.A.) 15 October 1986 cited in the application see examples	1-15
A	EP,A,0 517 565 (FIDIA S.P.A.) 9 December 1992 see claims	1-15
A	EP,A,0 341 745 (FIDIA S.P.A.) 15 November 1989 see claims	1

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/00294

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 10-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/EP 94/00294

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0526865	10-02-93	NONE		
EP-A-0462426	27-12-91	AU-B-	637235	20-05-93
		AU-A-	7806691	05-12-91
		CA-A-	2043527	02-12-91
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		JP-A-	62064802	23-03-87
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		WO-A-	8910941	16-11-89
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